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1 **Comparison of structural features and antioxidant activity of polysaccharides**

2 **from natural and cultured *Cordyceps sinensis***

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16 Running Title: Polysaccharide structure and antioxidant activity

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22

23

24 **Abstract**

25 Four polysaccharides (named as P1, P2, and P3 from three natural *Cordyceps sinensis*
26 and P4 from cultured *C. sinensis*) were obtained by hot-water extraction and ethanol
27 precipitation and their structural characteristics as well as antioxidant potentials were
28 compared. Results revealed that the backbone of P1, P2, and P3 comprised
29 α -1,4-glucose, with a branching point mainly at position 6 and terminating at glucose.
30 On the other hand, the structure of P4 was highly complex, mainly comprising
31 glucose, galactose, and mannose, with 1,4-glucose and 1,4-galactose as the main
32 chain. For *in vitro* antioxidant assays, all the four polysaccharides showed similar
33 scavenging capacity against DPPH and hydroxyl radicals, whereas P1 had a relative
34 low ferric reducing ability, possibly related to a combination of factors such as the
35 phenolic compounds and amino acids that conjugated in polysaccharides.

36

37

38 **Keywords**

39 Natural/cultured; *Cordyceps sinensis*; Polysaccharide; Structure; Antioxidant

40

41 **Introduction**

42 *Cordyceps* is a parasitic fungus that grows on the larva of insects. It has been reported
43 that there are more than 350 species belonging to the genus of *Cordyceps* all over the
44 world, out of which about 120 species have been found in China (1, 2). *C. sinensis*, in
45 particular, has been commonly known as a valued Chinese traditional herb for
46 hundreds of years. It was first recorded in “Ben-Cao-Cong-Xin” written by Yiluo Wu
47 in the Qing dynasty, but it was not known to the western society until the 17th century
48 (3). It is traditionally used in China for lung nourishing and kidney improvement as
49 well as relieving cough and reducing phlegm (4-6). However, it grows quite slowly
50 and requires a very specific habitat, resulting in limited productivity and very high
51 cost. Therefore, modern researchers have tried to isolate several mycelial strains from
52 wild *C. sinensis* and produced it using fermentation technology. To date, there are
53 more than 10 genera isolated from natural *C. sinensis*, and some of the fermented
54 products are manufactured on a large scale (7, 8).

55 Polysaccharide is considered to be one of the major bioactive constituents in both
56 natural and cultured *C. sinensis*. Pharmacological studies have found that
57 polysaccharides isolated from *C. sinensis* exhibit immunomodulatory, antitumor,
58 hypoglycaemic, and anti-fibrosis activities (9). The structure of polysaccharides, such
59 as Molecular weight (Mw), degree of branching, and chain conformation, were found
60 to be closely related to their biological activities (10, 11). However, little literature has
61 been devoted to comparing the chemical composition and structural features between
62 natural *C. sinensis* and its mycelium produced by fermentation. Currently, both

63 natural and cultured *C. sinensis* products are sold as healthy foods in South East Asia.
64 Whether the polysaccharide fractions have similar structural features and whether the
65 cultured mycelium could be regarded as a substitute of natural counterpart has
66 attracted research attention. In our previous study, differences in terms of amino acid
67 composition, minerals, as well as contents of adenine, adenosine, and mannitol, have
68 been observed not only between natural and cultured *C. sinensis* but also among
69 natural samples (12). It indicated that the originality and growth environment had an
70 impact on the chemical composition of *C. sinensis*.
71 In order to conduct a comprehensive and detailed evaluation among them, we first
72 evaluated the chemical composition and bioactive constituents' contents to compare
73 the bioactivities among them. Subsequently, we investigated polysaccharides with
74 regard to structural information and pharmacological properties. Therefore, in this
75 study, we isolated the polysaccharides from three natural and one cultured mycelium
76 of *C. sinensis* and determined the Mw, monosaccharide composition, and glycosidic
77 linkages in order to compare their structural characteristics. In addition, *in vitro*
78 antioxidant activity was studied for the purpose of exploiting new food sources of
79 antioxidants.

80 **Materials and Methods**

81 **Materials**

82 Natural *C. sinensis* were obtained from the Qinghai Province (Golog, Yushu, and
83 surrounding area of Xining) at different altitudes of above 4,800 m, above 4,200 m,
84 and between 2,800 and 3,300 m, named as S1, S2, and S3, respectively. Cultured *C.*

85 *sinensis* (named as S4) was purchased from Guoyao Company (Jiangxi, China).
86 T-series dextrans (T-10, T-40, T-70, T-500, and T-2000) were purchased from
87 Pharmacia Biotech (Uppsala, Sweden). Ascorbic acid, DPPH, TPTZ, and all the
88 monosaccharide standards including L-fucose (Fuc), L-rhamnose (Rha), D-arabinose
89 (Ara), D-galactose (Gal), D-glucose (Glc), D-mannose (Man), D-xylose (Xyl),
90 D-fructose (Fru), D-ribose (Rib), D-galacturonic acid (GalA), and D-glucuronic acid
91 (GlcA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium
92 borodeuteride (NaBD₄, 98 atom% D) was purchased from Acros Organics (Morris
93 Plains, NJ, USA). All other reagents were of analytical grade unless specified
94 otherwise.

95 **Preparation of polysaccharides**

96 Grounded natural samples and mycelium were soaked with 80% ethanol overnight to
97 remove pigment and other ethanol-soluble constituents, respectively. The residues
98 were then dried and extracted three times with hot water (95–100°C) for 2h.. All the
99 supernatant was collected and concentrated in a rotary evaporator at 55°C under
100 reduced pressure. The crude polysaccharides were obtained by precipitation with four
101 volumes of anhydrate ethanol and named as C1, C2, C3, and C4 corresponding to S1,
102 S2, S3, and S4, respectively.

103 The crude polysaccharides were further treated three times with Sevag reagent
104 (chloroform/1-butanol, v/v = 4:1) to remove proteins. Next it was dialyzed against tap
105 water for 48 h and distilled water for 24 h and finally lyophilized to give purified
106 polysaccharide fractions, namely P1, P2, P3, and P4 from C1, C2, C3, and C4,

107 respectively.

108 **Determination of molecular size distribution**

109 The molecular weight distributions of polysaccharides were determined by
110 high-performance gel-permeation chromatography (HPGPC) performed using an
111 Agilent 1260 LC instrument (Agilent Technologies Inc., Santa Clara, CA, USA)
112 equipped with a refractive index detector (RID; Agilent, Santa Clara, CA, USA), a
113 variable wavelength detector (VWD; Agilent, Santa Clara, CA, USA), and an
114 Ultrahydrogel™ Linear column (7.8 mm × 300 mm) (Waters Corp., Milford, MA,
115 USA). The polysaccharides were eluted with 0.1 M sodium chloride (dissolved in
116 0.02% NaN₃ aqueous solution) at a flow rate of 0.6 mL/min. The column temperature
117 was maintained at 35.0 ± 0.1°C. The polysaccharides were filtered through a 0.45-μm
118 membrane prior to injection. The Mw of the samples was calculated according to the
119 calibration curve established using dextran standards (T-10, T-40, T-70, T-500, and
120 T-2000).

121 **Identification of monosaccharide composition**

122 Monosaccharide composition was analyzed by high-performance anion-exchange
123 chromatography coupled with pulsed amperometric detection (HPAEC-PAD) on a
124 Dionex ICS-5000 System (Dionex Corp., Sunnyvale, CA, USA) equipped with a
125 CarboPac PA20 Guard (3 mm × 30 mm) and Dionex CarboPac PA20 column (3 mm ×
126 150 mm). Polysaccharides (10 mg) were hydrolyzed with 2 M sulfuric acid (H₂SO₄)
127 at 100°C for 6 h. The hydrolysate was diluted to suitable concentrations and filter
128 through 0.45-μm filters prior to injection and 10 μL of this solution was quantitatively

129 analyzed on the basis of monosaccharide standards. Separation was performed with
130 gradient elution comprising a mixture of distilled water, NaOH, and CH₃COONa (13).
131 Chromeleon software was used to control the instrument and process the data.

132 **Analysis of the FTIR spectra**

133 The FTIR spectrum of polysaccharides was analyzed on a Thermo Nicolet 5700
134 infrared spectrometer (ThermoElectron, Madison, WI, US) in the range of 4,000–400
135 cm⁻¹ using the KBr disc method.

136 **Methylation analysis**

137 The methylation procedure of polysaccharides was performed according to the
138 method by Ciucanu and Kerek (14) with minor modifications. In brief, approximately
139 3 mg of dried samples were completely dissolved in 0.5 mL anhydrous DMSO and
140 then dried NaOH powder (20 mg) was added to the solution. After stirring for 3 h, 1
141 mL iodomethane was added dropwise to the mixture in an ice bath. The reaction
142 mixture was further stirred under dark conditions for 2.5 h followed by addition of 0.2
143 mL water to stop the reaction. The methylated polysaccharides were extracted with
144 chloroform, passed through a sodium sulfate column to remove water, and finally
145 dried under a stream of nitrogen. Complete methylation was confirmed by the
146 disappearance of hydroxyl absorption in the FTIR spectrum. The methylated
147 polysaccharide was then hydrolyzed by 4 M trifluoroacetic acid, reduced with NaBD₄,
148 and acetylated with CH₃COOH to produce partially methylated alditol acetates
149 (PMAA), which were analyzed by gas chromatography- mass spectrometry
150 (GC–MS) (Agilent Technology 7890A/5975C, USA).

151 **Amino acid composition analysis**

152 The amino acid composition of polysaccharides was investigated by an automatic
153 amino acid analyzer (L-8900, Hitachi, Japan). Polysaccharides were hydrolyzed with
154 6 M HCl under nitrogen atmosphere at 110°C for 24 h. The resulting hydrolysates
155 were then evaporated and redissolved in water and filtered through a 0.22-μm
156 membrane prior to analysis.

157 **Determination of total phenolic content**

158 The total phenolic content of polysaccharides was measured according to the
159 Folin–Ciocalteu colorimetric method (15), with slight modifications. In brief, 1 mL
160 polysaccharide solution, 1 mL Folin–Ciocalteu reagent, and 3 mL 10% Na₂CO₃
161 solution were mixed and further diluted to 10 mL. After incubation at 25 °C under
162 dark conditions for 30 min, the absorbance of the solution was measured at 764 nm.
163 Different concentrations of gallic acid (0.01–0.05 mg/mL) were used as a standard
164 curve and total phenolic content of polysaccharides was presented as gallic acid
165 equivalents (GAE) in milligrams per gram of dry weight polysaccharide.

166 **Antioxidant activities**

167 ***DPPH radical scavenging activity***

168 The scavenging activity of polysaccharides against DPPH radical was determined as
169 described by Mao *et al.* (16) with slight modification. In brief, 2 mL of different
170 concentrations of polysaccharides (0.25–4 mg/mL) were mixed with equal volumes of
171 DPPH-ethanol solution and incubated for 30 min at room temperature. The mixture
172 was measured at 517 nm with ascorbic acid as a positive control, and the scavenging

173 percentage was calculated by the following equation:

174
$$\text{Scavenging activity (\%)} = (1 - (A_s - A_{s0})/A_0) \times 100,$$

175 where A_0 , A_s , and A_{s0} are the absorbance of DPPH reagent, polysaccharide, and
176 polysaccharide without DPPH, respectively.

177 ***Hydroxyl radical scavenging activity***

178 The hydroxyl radical scavenging activities of polysaccharides were determined
179 according to the method of Gao *et al.* (17) with minor modification. In brief, 1 mL
180 polysaccharide solutions with different concentrations (0.25–4 mg/mL) were mixed
181 with 1 mL $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (9 mM), 1 mL salicylic acid-ethanol (9 mM), and 1 mL H_2O_2
182 (9 mM). Then the mixture was incubated at 37 °C for 30 min and the absorbance was
183 measured at 510 nm. Distilled water and ascorbic acid were used as a blank and
184 positive control, respectively. The ability to scavenge hydroxyl radicals was calculated
185 according to the following equation:

186
$$\text{Scavenging activity (\%)} = (1 - (A_s - A_{s0})/A_0) \times 100,$$

187 where A_0 , A_s , and A_{s0} are the absorbance of the blank (water instead of
188 polysaccharide), sample, and control (water instead of H_2O_2) groups, respectively.

189 ***The ferric reducing ability of plasma (FRAP)***

190 The total antioxidant potential of polysaccharides was assayed by the method of
191 Benzie and Strain (18). FRAP reagent was comprised 300 mM acetate buffer (pH 3.6),
192 10 mM TPTZ, and 20 mM FeCl_3 solution in a ratio of 10:1:1. A 300 μL freshly
193 prepared FRAP stocking solution was pre-warmed to 37°C for 10 min, and a reagent
194 blank was recorded at 593 nm. Then 30 μL of distilled water and 10 μL of

195 polysaccharide solutions (0.25–4 mg/mL) was added to the FRAP stocking solution
196 under dark conditions and a second reading was taken after 30 min. Different
197 concentrations of FeSO₄ solution ranging from 100 to 1,000 µM were used to
198 establish a standard curve. The antioxidant capacity of each sample was expressed as
199 µmol Fe (II)/g dry weight of polysaccharide.

200 **Statistical analysis**

201 All assays were performed at least in triplicate. Data were analyzed by one-way
202 analysis of variance (ANOVA) using SPSS 17.0 software (SPSS Inc., Chicago, IL,
203 USA). Tukey's HSD test was used to determine the significant difference at a level of
204 $P < 0.05$.

205 **Results and Discussion**

206 **Extraction and molecular weight distribution**

207 The yields of crude polysaccharide extracted from three natural *C. sinensis* and
208 cultured *C. sinensis* were 9.43%, 9.78%, 8.94%, and 14.32% by weight, respectively.
209 After removal of protein by Sevag method, the subsequent yields were 27.58%,
210 31.57%, 38.30%, and 49.36% by weight, respectively.

211 HPGPC results revealed that the three natural polysaccharides exhibited similar
212 elution profiles (data not shown), indicating that there were no differences in
213 molecular weight distribution among polysaccharides from natural *C. sinensis*. As
214 shown in Fig. 1 (A), the molecular weight distribution profile of polysaccharide from
215 natural material mainly comprised three fractions with Mw of approximately 950 K,
216 15 K, and 1,000 Da, respectively. The peak at the retention time of 18.5 min (from RI

217 signal) was considered to be the existence of low molecular residues such as
218 monosaccharides and oligosaccharides. These residues were successfully removed
219 through dialysis treatment. After treating with Sevag reagent, most of the fraction that
220 had the highest UV signal at 280 nm was removed, and the fraction with a high Mw
221 remained dominant. On the contrary, the polysaccharide from cultured *C. sinensis*, P4,
222 presented a broad distribution profile with a shoulder peak as shown in Fig. 1 (B). The
223 average Mw was much lower compared to P1, P2 and P3, approximately 28 KDa. The
224 result showed that there was no significant difference in the molecular weight
225 distribution of P1, P2, and P3, and the major fraction was a high-molecular-weight
226 polymer, but it was completely different from P4.

227 **Monosaccharide composition**

228 After complete acid hydrolysis, the monosaccharide compositions of polysaccharides
229 from natural and cultured *C. sinensis* were analyzed by HPAEC-PAD. As shown in
230 Table 1, P1, P2, and P3 were all mainly composed of Glc with a small amount of Gal
231 and Man, although the percentages of Glc varied slightly, ranging from 81.72% to
232 89.22% for the crude polysaccharides and from 78.83% to 84.05% for deproteinated
233 polysaccharides, respectively. On the other hand, P4 had a much complicated
234 monosaccharide composition and mainly comprised Gal, Glc, and Man with a
235 percentage of 36.40%, 28.99%, and 24.81%, respectively. Besides, it also contained a
236 small amount of Ara (3.34%) and GalA (7.55%). Therefore, the result revealed a
237 rather distinct difference between polysaccharides from natural and cultured *C.*
238 *sinensis*.

239 **FTIR spectra**

240 All the polysaccharide exhibited a broad intense characteristic peak at approximately
241 $3,380\text{ cm}^{-1}$ for the hydroxyl group, a weak band at near $2,930\text{ cm}^{-1}$ that was attributed
242 to C–H stretching, and a group of absorptions at $1,400\text{--}1,200\text{ cm}^{-1}$ for the C–H
243 bending vibration (Fig. 2). The absorption band centered at approximately $1,650\text{ cm}^{-1}$
244 was caused by associating with water. P4 had weak absorption at $1,745\text{ cm}^{-1}$,
245 indicating the presence of carboxyl groups, which was consistent with the results of
246 monosaccharide composition analysis. Furthermore, a characteristic band at 887 cm^{-1}
247 was noticed in P4, indicating the existence of a β -configuration of sugar units,
248 whereas in P1, P2 and, P3 absorption was observed at approximately 845 cm^{-1}
249 corresponding to α -configuration. As expected, the FTIR spectra of P1, P2, and P3
250 were similar, but they had some differences from that of P4.

251 **Methylation analysis**

252 Methylation analysis revealed that all the three natural polysaccharides contained
253 similar types of glycosidic linkages, mostly 1→4 linkage pattern with branching
254 points at position 6 and terminating at glucose (Table 2). A small amount of terminal
255 and 1→3 linked Glc as well as 1→2 linked Man were also identified in the three
256 samples, whereas P1 also contained a small amount of 1→4 linked Gal and 1→6 and
257 1→4,6 linked Man. However, a small amount of terminal and 1→3,6 linked Gal as
258 well as 1→2,3 linked Man was only found in P2 and P3. On the other hand, the
259 results revealed P4 to be highly complex and that it could be a mixture of two or more
260 polysaccharide fractions. 1→4 linked Glc and 1→4 linked Gal may be part of the

261 main chain of P4. Man units were found as 1→2, 1→6, 1→2,6, and 1→4,6 linkages.
262 Ara was found as terminal and 1→5 linked residues. In addition, terminally linked
263 Gal as well as terminally 1→3 and 1→4, 6 linked Glc was presented in P4. On the
264 basis of these results, it was possible to conclude that the repeating unit of
265 polysaccharides from natural *C. sinensis* contained a backbone mainly comprising
266 1→4 linked Glc residues with branches attached to O-6 of some Glc. Although
267 15.44% of this linkage was found in P4, the content of 1→4,6 linked Glc was relative
268 low with only 1.42%. Furthermore, the branching point of P4 may possibly occur in
269 Man residues with 1→2,6 and 1→4,6 linkages. This finding obviously revealed the
270 similarity of glycosidic linkages among polysaccharides from three natural *C. sinensis*,
271 despite the different growth environments that mainly referred to diversity of altitude.
272 Another meaningful finding of this study was the distinctive differences of linkage
273 patterns between natural and cultured materials. Unlike the observed variations of
274 chemical composition reported previously (12), structure of the polysaccharide moiety
275 was not influenced by the growing habitats for natural *C. sinensis* collected from the
276 Qinghai Province. However, the polysaccharide from cultured *C. sinensis* was
277 completely different from the natural ones on the basis of the sugar residues and
278 glycosidic linkages.

279 **Amino acid composition**

280 The amino acid composition of polysaccharides from natural and cultured *C. sinensis*
281 was investigated (Table 3). There were 17 kinds of amino acids contained in P2 and
282 P3, whereas P1 did not contain tyrosine and P4 did not contain methionine. However,

the quantities of these two amino acids were not very high in the other polysaccharides, which were 0.18, 0.18, and 0.36 mg/g of tyrosine and 1.19, 0.90, and 1.19 mg/g of methionine for P2, P3, and P4, respectively. All four polysaccharides were abundant in glutamic acid, aspartic acid, and proline, and the content was the lowest in P4. Moreover, there were relatively high contents of threonine and serine, indicating the possible existence of O-glycosidic linkages.

Total phenolic content

Phenolic compounds, including phenolic acid, polyphenols, monophenols, and other phenolic derivatives, have a strong ability to scavenge free radical and potentially prevent oxidative stress-related diseases (19, 20). Although the extraction process did not aim to isolate phenolic compounds, the polysaccharides obtained from all four materials appeared to contain a certain amount of phenolic compounds, as shown in Fig. 3 (A). The total phenolic content of P1, P2, and P3 was 3.22 ± 0.05 , 5.16 ± 0.02 , and 7.63 ± 0.26 mg GAE/g, respectively. P4 presented a total phenolic level of 7.05 ± 0.46 mg GAE/g, showing no significant difference with the value of P3, but this level was significantly different from that of P1 and P2. A significant difference among the phenolic contents of the three natural *C. sinensis* was also observed, which can probably be attributed to the different habitats because factors such as moisture, altitude, and temperature influence the composition and content of phenolic compounds.

Antioxidant capacity

Reactive oxygen species (ROS) are commonly produced in the human body and their

305 excessive generation leads to oxidative stress. Deactivation of radicals occurs via two
306 major mechanisms: single electron transfer and hydrogen atom transfer (21).
307 Therefore, the *in vitro* antioxidant activity of polysaccharides from natural and
308 cultured *C. sinensis* was evaluated through three methods, which are compared with a
309 standard antioxidant (ascorbic acid). As shown in Fig. 3 (B), all four polysaccharides
310 displayed significant scavenging effect against DPPH radical in a dose-dependent
311 manner. However, they were found to exhibit low effect on scavenging hydroxyl
312 radicals compared to ascorbic acid, as shown in Fig. 3 (C). The polysaccharide from
313 cultured mycelium was the most effective of them and scavenged only 40% of
314 hydroxyl radicals. The FRAP values of the four polysaccharides increased in the order
315 of P3 > P4 > P2 > P1 with values of 56.79, 53.74, 44.51, and 30.19, respectively (Fig.
316 3 (D)), corresponding to the trends of total phenolic contents, indicating that the
317 observed antioxidant potential may be related to the phenolic constituents.

318 Natural polysaccharides have been reported to always be conjugated with other
319 constituents, such as amino acid, protein, lipids, and phenolic compounds (22).
320 Positive correlation between antioxidant actions and the total phenolic or protein
321 content in the polysaccharide has been observed in several reports (23, 24). The
322 antioxidant activity of protein molecules, in general, may be attributed to the amino
323 acid because several amino acids, such as tyrosine, methionine, histidine, lysine, and
324 tryptophan, are proved to be capable of donating protons to electron-deficient radicals
325 (25-27). In our previous study, we compared the antioxidant activity of water extract
326 among four samples (12). Results showed that cultured *C. sinensis* had relatively

327 more effective abilities against scavenging DPPH and hydroxyl radicals as well as a
328 higher reducing ferrous power than natural *C. sinensis*, but these effects were not
329 significantly different among the three natural samples. After increasing the
330 polysaccharide purity by ethanol precipitation and Sevag reagent treatment, these
331 antioxidant activities did not improve significantly. On the other hand, although the
332 content of protein and total phenolic decreased dramatically in P1, P2, P3, and P4
333 compared with these water extracts, the effects did not appear to reduce significantly.
334 These findings indicated that the antioxidant activity of polysaccharides from *C.*
335 *sinensis* might be associated with a combination of factors because the
336 polysaccharides always contained certain amount of other antioxidative constituents.

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345 **Statement of Human and Animal Rights**

346 This study did not involve any experimentation with human or animal subjects
347 performed by any of the authors.

348 **Disclosure**

349 Wang, Nie, Kan, Chen, Cui, Phillips, Phillips, and Xie declare that they have no
350 conflict of interest.

351

352 **References**

- 353 1. Jiang Y, Yao Y. Current understanding of molecular systematics of *Cordyceps*.
354 J. Fungal Res. 2: 58-67 (2004).
- 355 2. Sung JM, Lee HK, Yang KJ. Classification of *Cordyceps spp.* by
356 morphological characteristics and protein banding pattern. Korean J. Mycol.
357 23: 92-104 (1995).
- 358 3. Li SP, Yang FQ, Tsim KWK. Quality control of *Cordyceps sinensis*, a valued
359 traditional Chinese medicine. Pharmaceut. Biomed. 41: 1571-1584 (2006).
- 360 4. Chen PX, Wang SN, Nie SP, Marcone M. Properties of *Cordyceps sinensis*: A
361 review. J. Funct. Foods. 5: 550-569 (2013).
- 362 5. Zhu JS, Halpern GM, Jones K. The scientific rediscovery of an ancient
363 Chinese herbal medicine: *Cordyceps sinensis* Part I. J. Altern. Complem. Med.
364 4: 289-303 (1998).
- 365 6. Zhu JS, Halpern GM, Jones K. The scientific rediscovery of a precious ancient
366 Chinese herbal regimen: *Cordyceps sinensis* Part II. J. Altern. Complem. Med.
367 4: 429-457 (1998).
- 368 7. Liang ZQ. Current situation and ponderation of *Cordyceps* Fr. research and
369 exploitation in China. Acta Edulis Fungi. 2: 53-62 (2001).
- 370 8. Yan JK, Wang WQ, Wu JY. Recent advances in *Cordyceps sinensis*
371 polysaccharides: Mycelial fermentation, isolation, structure, and bioactivities:
372 A review. J. Func. Foods. 6: 33-47 (2014).
- 373 9. Nie SP, Cui SW, Xie MY, Phillips AO, Phillips GO. Bioactive polysaccharides

- 374 from *Cordyceps sinensis*: Isolation, structure features and bioactivities. Bioact.
375 Carbohyd. Dietary Fibre. 1: 38-52 (2013).
- 376 10. Tao YZ, Zhang LN, Cheung PC. Physicochemical properties and antitumor
377 activities of water-soluble native and sulfated hyperbranched mushroom
378 polysaccharides. Carbohyd. Res. 341: 2261-2269 (2006).
- 379 11. Zhang LN, Li XL, Xu XJ, Zeng FB. Correlation between antitumor activity,
380 molecular weight, and conformation of lentinan. Carbohyd. Res. 340:
381 1515-1521 (2005).
- 382 12. Wang JQ, Kan LJ, Nie SP, Chen HH, Cui SW, Phillips AO, Phillips GO, Li YJ,
383 Xie MY. A comparison of chemical composition, bioactive components and
384 antioxidant activity of natural and cultured *Cordyceps sinensis*. LWT-Food Sci.
385 Techn. 63: 2-7 (2015).
- 386 13. Dai J, Liang L, Yin H. Analysis of monosaccharide compositions in
387 polysaccharides from *D. salina* by high-performance anion-exchange
388 chromatography. Food Ferment. Ind. 32: 131-135 (2006).
- 389 14. Ciucanu I, Kerek F. A simple and rapid method for the permethylation of
390 carbohydrates. Carbohyd. Res. 131: 209-217 (1984).
- 391 15. Singleton V, Orthofer R, Lamuela-Raventos R. Analysis of total phenols and
392 other oxidation substrates and antioxidants by means of folinciocalteu reagent.
393 Method Enzymol. 299: 152-178 (1999).
- 394 16. Mao GH, Zou Y, Feng WW, Wang W, Zhao T, Ye CW, Zhu Y, Wu XS, Yang
395 LQ, Wu XY. Extraction, preliminary characterization and antioxidant activity

- 396 of Se-enriched Maitake polysaccharide. Carbohydr. Polym. 101: 213-219
397 (2014).
- 398 17. Gao CJ, Wang YH, Wang CY, Wang ZY. Antioxidant and immunological
399 activity in vitro of polysaccharides from *Gomphidius rutilus* mycelium.
400 Carbohydr. Polym. 92: 2187-2192 (2013).
- 401 18. Benzie IF, Strain J. The ferric reducing ability of plasma (FRAP) as a measure
402 of “antioxidant power”: the FRAP assay. Anal. Biochem. 239: 70-76 (1996).
- 403 19. Fogarasi AL, Kun S, Tankó G, Stefanovits-Bányai É, Hegyesné-Vecseri B. A
404 comparative assessment of antioxidant properties, total phenolic content of
405 einkorn, wheat, barley and their malts. Food Chem. 167: 1-6 (2015).
- 406 20. Weng CJ, Yen GC. Chemopreventive effects of dietary phytochemicals against
407 cancer invasion and metastasis: phenolic acids, monophenol, polyphenol, and
408 their derivatives. Cancer Treat. Rev. 38: 76-87 (2012).
- 409 21. Niu YG, Shang PP, Chen L, Zhang H, Gong L, Zhang XW, Yu WJ, Xu YH,
410 Wang Q, Yu LL. Characterization of a novel alkali-soluble
411 heteropolysaccharide from tetraploid *Gynostemma pentaphyllum* makino and
412 its potential anti-inflammatory and antioxidant properties. J. Agric. Food
413 Chem. 62: 3783-3790 (2014).
- 414 22. Chen H, Zhang M, Qu Z, Xie B. Antioxidant activities of different fractions of
415 polysaccharide conjugates from green tea (*Camellia Sinensis*). Food Chem.
416 106: 559-563 (2008).
- 417 23. Wang J, Hu S, Nie S, Yu Q, Xie M. Reviews on mechanisms of in vitro

- 418 antioxidant activity of polysaccharides. *Oxid. Med. Cell Longe.* 2016 (2015).
- 419 24. Zhang H, Cui SW, Nie SP, Chen Y, Wang YX, Xie MY. Identification of
420 pivotal components on the antioxidant activity of polysaccharide extract from
421 *Ganoderma atrum*. *Bioact. Carbohydr. Dietary Fibre.* 7: 9-18 (2016).
- 422 25. Elias RJ, Kellerby SS, Decker EA. Antioxidant activity of proteins and
423 peptides. *Crit. Rev. Food Sci.* 48: 430-441 (2008).
- 424 26. Minelli A, Bellezza I, Grottelli S, Galli F. Focus on cyclo (His-Pro): history
425 and perspectives as antioxidant peptide. *Amino Acids.* 35: 283-289 (2008).
- 426 27. Pérez RA, Iglesias MT, Pueyo E, González M, de Lorenzo C. Amino acid
427 composition and antioxidant capacity of Spanish honeys. *J. Agric. Food Chem.*
428 55: 360-365 (2007).

Figure Captions

Figure 1. HPGPC profiles of the polysaccharides from (A) natural *Cordyceps sinensis* and (B) cultured mycelium monitored by RID (straight line) and VWD (dotted line) at 280 nm.

Figure 2. FTIR spectrum of the polysaccharides.

Figure 3. (A) Total phenolic contents of polysaccharides calculated as mg GAE/g. The scavenging activities of polysaccharides on (B) DPPH and (C) hydroxyl radicals. (D) Ferric reducing ability of polysaccharides. Data is expressed as the mean \pm SD of triplicate determinations and different letters represent significant differences ($P < 0.05$).

Tables

Table 1

Monosaccharide compositions of the polysaccharides ^a

Sample	Ara, %	Gal, %	Glc, %	Man, %	GalA, %
C1	ND ^b	3.44	88.01	8.55	ND
C2	ND	3.04	81.72	15.24	ND
C3	ND	2.62	89.22	8.16	ND
C4	trace	38.48	31.99	23.15	6.38
P1	ND	7.07	80.92	12.00	ND
P2	ND	4.83	84.05	11.12	ND
P3	ND	6.14	78.83	15.03	ND
P4	3.34	36.40	28.99	24.81	7.55

^a calculated as a molar percentage.

^b “ND” means not detected.

Table 2

The glycosidic linkages of the polysaccharides determined by GC–MS

Glycosyl residues	Linkage patterns	Peak area percentage (%)			
		P1	P2	P3	P4
Ara	t-Araf	ND ^a	ND	ND	1.91
	5-Araf	ND	ND	ND	3.24
Glc	t-Glcp	11.60	15.05	13.70	10.26
	3-Glcp	5.07	2.23	2.52	5.85
	4-Glcp	51.59	58.28	60.55	15.44
	6-Glcp	ND	0.81	0.84	ND
	3,6-Glcp	1.62	0.60	ND	ND
	4,6-Glcp	9.11	12.59	11.42	1.42
	t-Galf	ND	1.61	1.17	ND
	t-Galp	ND	ND	ND	6.63
Gal	4-Galp	8.48	1.71	0.97	22.54
	3,6-Galp	ND	1.54	1.41	ND
Man	2- Manp	3.51	3.02	2.95	5.17
	6- Manp	2.61	0.42	ND	7.20
	2,3- Manp	ND	2.15	2.63	ND
	2,6- Manp	0.82	ND	ND	3.11
	4,6- Manp	2.89	ND	ND	9.20

^a “ND” means not detected.

Table 3

Amino acid composition of the polysaccharides

Amino acid (mg/g)	P1	P2	P3	P4
Aspartic acid	10.45	17.57	8.32	8.45
Threonine ^a	10.48	11.55	9.17	7.15
Serine	9.25	9.51	10.40	4.62
Glutamic acid	20.97	22.58	18.32	10.08
Glycine	11.79	10.89	9.91	5.56
Alanine	9.89	10.82	9.49	4.86
Cystine	1.57	1.45	1.57	0.24
Valine ^a	9.49	8.32	10.07	3.98
Methionine ^a	1.19	0.90	1.19	ND ^b
Isoleucine ^a	4.20	4.20	4.98	1.44
Leucine ^a	5.31	6.43	7.48	1.44
Tyrosine	ND ^b	0.18	0.18	0.36
Phenylalanine ^a	3.30	2.97	3.63	1.16
Lysine ^a	12.72	11.26	13.08	3.95
Histidine	6.51	5.43	9.15	2.02
Arginine	10.71	9.41	14.46	2.61
Proline	23.20	13.93	24.35	6.91
total amino acids (TAA)	151.03	147.39	155.77	64.82
total essential amino acids (EAA)	46.70	45.62	49.62	19.12
EAA/TAA (%)	30.92	30.95	31.86	29.49

^a means essential amino acid.^b “ND” means not detected.

Figures

Figure 1

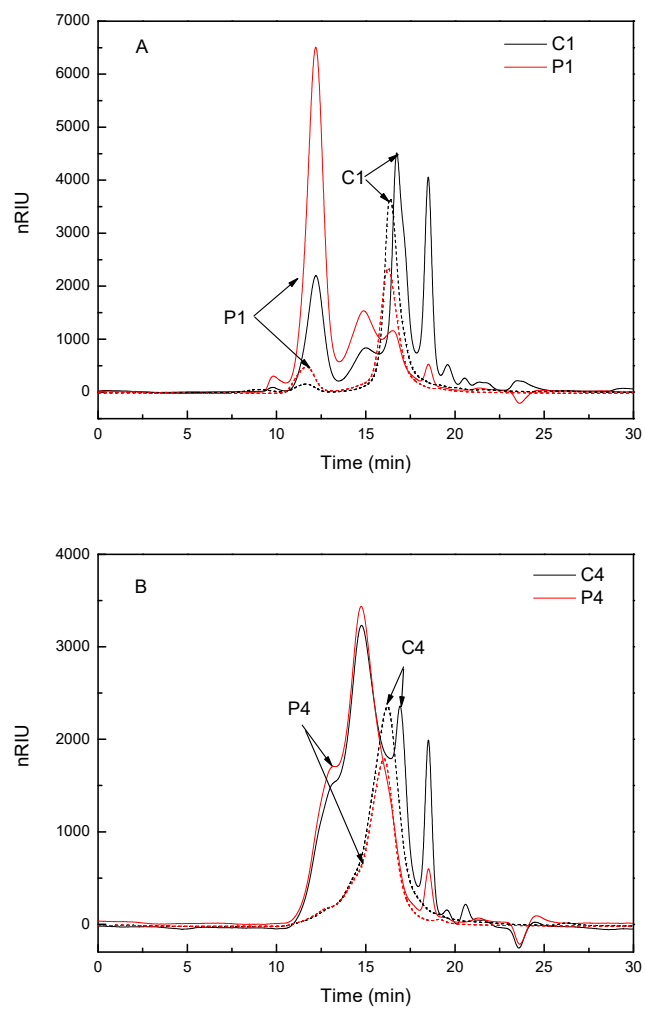


Figure 2

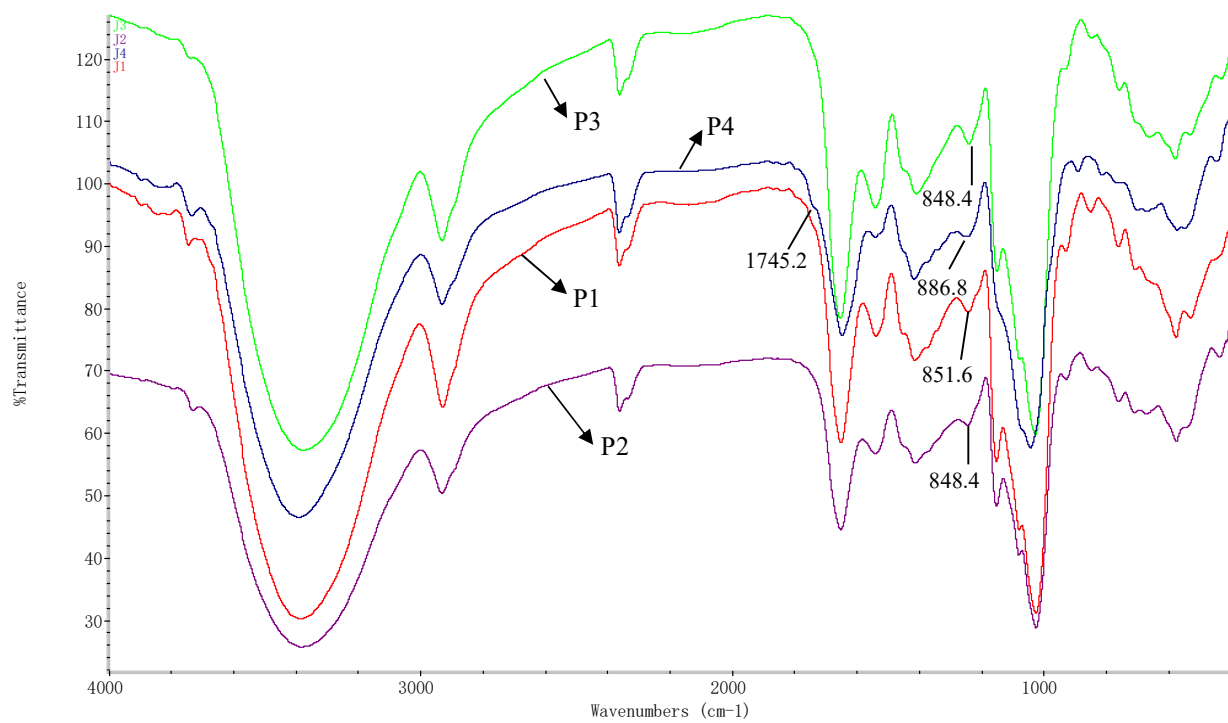


Figure 3

